# *OPTICAL ROTATORY DISPERSION OF HUMAN CARBONIC ANHYDRASES: COTTON EFFECTS AND AROMATIC ABSORPTION BANDS**

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Three distinct carbonic anhydrases, now denoted as A, B, and C, have been separated from human erythrocytes, by several different methods.[1-4] Enzyme A is present in small amount and with low specific activity, B in much larger amount but also with low specific activity, C in small amount but with high specific activity.[5] All three enzymes have molecular weights close to 30,000 and each contains one atom of zinc per molecule. In what follows, we are chiefly concerned with enzymes B and C.

Studies of optical rotatory disperson, short wavelength ultraviolet absorption, and infrared spectra have suggested that native carbonic anhydrase B, although a highly compact molecule with a very low intrinsic viscosity, contains little or no $\alpha$-helix.[3] Similar evidence from rotatory dispersion, some of which we report here, is now available for carbonic anhydrase C. In recent studies, we have also observed unusual features in the optical rotatory dispersion of both these enzymes, involving Cotton effects in the region between 260 and 300 m$\mu$, which are evidently associated with the absorption bands of the aromatic amino acid residues in the same region. These Cotton effects disappear on denaturation of these enzymes in acid, urea, or guanidine hydrochloride. Although somewhat similar Cotton effects in this range of wavelengths have been found in poly-L-tyrosine and in a few proteins, as we point out later in the discussion, the rotatory dispersion patterns of the carbonic anhydrases are highly specific. We report here some major features of the observed effects, with comments on their implications for the structure of these enzymes.

*Methods.—Experimental:* The carbonic anhydrases were prepared from human erythrocytes supplied by the Protein Foundation Laboratories, Jamaica Plain, Massachusetts, through the kindness of Dr. R. B. Pennell. We removed hemoglobin from the carbonic anhydrases by treatment with ethanol and chloroform at low temperature.[3] However, we subsequently separated the different carbonic anhydrases, not on hydroxylapatite columns as previously,[3] but by passage through a column of diethyl-aminoethyl Sephadex (DEAE-Sephadex A-50, medium grade, obtained from Pharmacia, Inc., New York) in tris(hydroxymethyl)aminomethane buffer at pH 8.7–8.9. The method is still undergoing modification and will later be described in detail; it involves either gradual or stepwise increase of the buffer concentration at nearly constant pH. Carbonic anhydrase C, with an isoelectric point of 7.3, is eluted first; enzyme B, which is isoelectric at pH 5.7, emerges at a somewhat higher ionic strength; and enzyme A emerges later, mixed with some inactive components, at still higher ionic strength. The high capacity of the DEAE-Sephadex permits preparation of much larger quantities of the enzymes, in a single run, than we could obtain by the earlier procedure[3] which employed hydroxylapatite columns; quantities of 6 gm or more of crude carbonic anhydrase have been separated on a DEAE-Sephadex column with a total volume of 2.3 liters, at a flow rate of about 3 ml per minute. This yields several grams of enzyme B, which is sufficiently pure to give only a single band on starch gel electrophoresis by procedures previously described.[3] Its specific activity, assayed by the procedure of Wilbur and Anderson,[7] was constant in different fractions of the separated band of eluted material at 14,000 enzyme units per mg protein. The enzyme C fraction, on the other hand, although it gave a specific activity of 33,000 units per mg, yielded electrophoretic patterns containing a minor band indicating the presence of some residual enzyme B. We removed this by

selective adsorption of enzyme C on sulfoethyl-Sephadex[6] at pH 6 in 0.005 *M* sodium phosphate buffer, and its subsequent elution with 0.1 *M* phosphate buffer at pH 8.9. It was necessary to repeat this operation a second time to obtain enzyme C which migrated as a single band on starch gel. The resulting preparations of enzyme C gave excellent crystals in concentrated ammonium sulfate solutions.

After two months at 4° in concentrated ammonium sulfate solutions, both carbonic anhydrases B and C were still electrophoretically homogeneous and showed no loss of enzyme activity.

We are indebted to Dr. P.-O. Nyman of the University of Göteborg, Sweden, for his kindness in supplying samples of human carbonic anhydrases A, B, and C, as prepared by the methods previously described by him.[1, 4]

We prepared guanidine hydrochloride from guanidine carbonate (Eastman White Label) by the method of Anson.[8] The purified material showed almost no absorption in the ultraviolet down to 230 mμ. We recrystallized urea (Merck reagent grade) twice from cold 95% ethanol, and prepared urea solutions from the crystals immediately before use.

We calculated protein concentrations from ultraviolet absorption measurements at 280 mμ, taking $A_{280}$, for a solution containing 10 mg protein per ml, as 16.3 for enzyme B and 17.8 for enzyme C.[1, 3] If the figure of 18.7 for enzyme C, reported more recently by Nyman and Lindskog,[4] were to be accepted, it would be necessary to multiply all our reported values of the mean residue rotation [R'] of enzyme C by the factor 1.05.

Our earlier measurements of optical rotation were made between 334 and 578 mμ in the laboratory of Prof. Paul Doty on a Rudolph model 200S polarimeter equipped with a Beckman DU monochromator. Later measurements, extending down to 220 mμ, were made on a Cary model 60 recording spectropolarimeter, for the use of which we are indebted to Prof. E. R. Blout. Measurements on the Rudolph instrument were made in a 1-dm cell, on the Cary instrument in several cells with path lengths from 0.5 to 0.001 dm. Blank readings were taken on all the cells, over the range of wavelengths studied, and subtracted from the measurements on the protein solutions. However, as Iizuka and Yang[9] have emphasized, the positioning of the cell on the holder in the Cary apparatus is of critical importance, and may shift the baseline by as much as 0.01°. Slight stresses may also affect the blank readings of the cells significantly. We therefore found that readings on the same solution in different cells did not always give consistent results; the dispersion patterns remained the same, but the absolute values of specific rotation at a given wavelength were sometimes variable. Especially in the region below 240 mμ it was necessary to use cells of very short path length, because of the high absorption of the protein solutions. Under these circumstances, because of the low specific rotation of the proteins, the observed rotations were often less than 0.01°, and the contribution of the blank was sometimes as high as 40% of the measured value. At longer wavelengths the contribution of the blank was less than 10%, and often much less than this.

The measured and corrected rotations were converted to specific rotations, $[\alpha]_\lambda$, and then into values of the reduced mean residue rotation, [R'], at wavelength λ by the formula:

$$[R']_\lambda = \left(\frac{3}{n^2 + 2}\right)\left(\frac{W_m}{100}\right)[\alpha]_\lambda. \quad (1)$$

Here $n$ is the refractive index of the solvent, and $W_m$ is the mean residue weight of the protein which is found to be 114 for both carbonic anhydrases from amino acid analyses.[3, 4] We made use of a table of numerical values for the refractive index of water from 700 to 200 mμ, calculated by the equation of Duclaux and Jeantet, as given by Dorsey.[10] Fasman[11] has now published a similar table. We assumed the refractive indices of 0.1 *M* phosphate buffers and 0.1 *M* phosphoric acid solutions to be identical with those of pure water. We took the values of $n$ for 8 *M* urea solutions from the compilation of J. Foss, Y. Kang, and J. A. Schellman, recently published in the review by Fasman (ref. 11, p. 957), and calculated values of $n$ at lower concentrations of urea by the Lorentz-Lorenz equation; to calculate the densities of the urea solutions we assumed a partial specific volume of 0.738 for urea.

We are indebted to Dr. K. Hamaguchi of Osaka University for informing us of his refractive index measurements at 546 mμ on guanidine hydrochloride solutions; these gave values of 1.3648 (at 1.26 *M*), 1.3858 (2.80 *M*), 1.4031 (3.78 *M*), and 1.4232 (4.98 *M*), at pH 7.0, 25°, in 0.01 *M* ethylenediamine tetraacetate. Hamaguchi and Kurono[12] have previously employed these data

in computing [R'] values for lysozyme solutions. In calculating dispersions we have assumed that a guanidine hydrochloride solution will have the same dispersion as a urea solution if both solutions have the same refractive index at 546 mμ.

*Results.*—Figure 1 represents [R'] as a function of wavelength, from 600 to approximately 220 mμ for enzyme B, both in the native state at pH 7.0 and in acid at pH 1.6. Figure 2 presents similar data for enzyme C. The data, particularly those below 300 mμ, present some unusual features. The numerical values of $-[R']$ are small compared to those of many helical synthetic polypeptides, and of several proteins that have been studied.[13–15] The observed minima in the curves for the native proteins lie at 223–224 $m\mu$ for enzyme B, and at 227 $m\mu$ for enzyme C. On acid denaturation the minima shift to 229–230 mμ for both enzymes, and [R'] at the minimum becomes considerably more negative, although the numerical values of [R'] remain far smaller than those characteristic of helical polypeptides at this

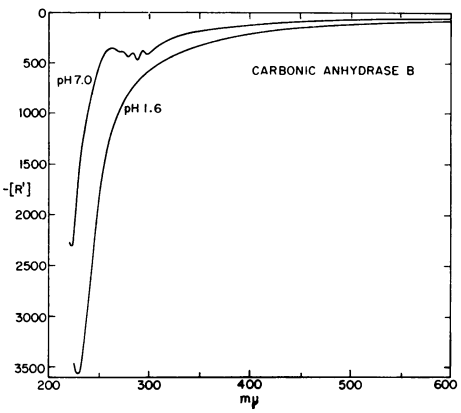


FIG. 1.—Rotatory dispersion of carbonic anhydrase B. Molar residue rotation $-[R']$, plotted against wavelength. Native protein at pH 7.0 in 0.1 *M* phosphate buffer. Acid-denatured protein in 0.1 *M* $H_3PO_4$, pH 1.6. The minimum in the curve for the native protein is at $\lambda_{min}$ 223 mμ, [R'] = −2320°; for the acid-denatured protein $\lambda_{min}$ = 229 mμ, [R'] = −3570°.

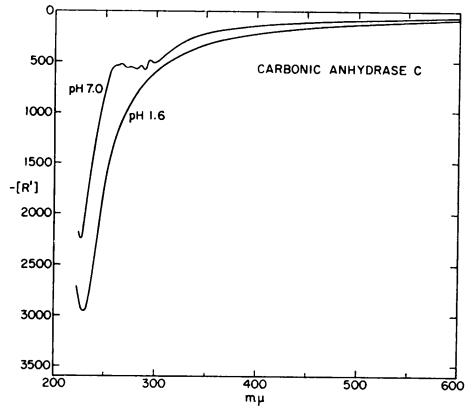


FIG. 2.—Rotatory dispersion of carbonic anhydrase C. Solvents employed were the same as in Fig. 1. At pH 7.0, $\lambda_{min}$ = 227 mμ, [R'] = −2240°. At pH 1.6, $\lambda_{min}$ = 229 mμ, [R'] = −2970°.

wavelength. Likewise, the positions of the minima are well below the value of 233 mμ which is characteristic of the trough of the Cotton effect in helical polypeptides and in several proteins that are generally presumed to be at least partly helical. Unfortunately, the absorption of the enzyme solutions becomes so high at short wavelengths that we have not yet been able to make reliable measurements below about 220 mμ.

The most striking feature of the data in Figures 1 and 2, however, is the presence of Cotton effects in the native proteins in the region between 260 and 300 mμ. As the figures show, the rather complicated dispersion pattern in this region which is characteristic of the native proteins disappears on denaturation in acid and is replaced by a smooth featureless dispersion curve which is shifted to much more negative values of [R'] than those characteristic of the native protein in this region. Figures 3 and 4 show the pattern of these Cotton effects in considerably more detail, and also show the effects of dissolving the enzymes in guanidine hydrochloride at various concentrations from 0.5 to 4 *M*.

The question immediately arises whether the dispersion curves for the native proteins in Figures 3 and 4 represent genuine Cotton effects or artifacts. The recorded effects, especially between 270 and 290 mμ, coincide with the aromatic absorption bands of these enzymes, which are intense because of their high content of aromatic amino acid residues (Table 1). Urnes and Doty[16] have pointed out how readily ar-

TABLE 1

AROMATIC AMINO ACID CONTENT OF CARBONIC ANHYDRASES B AND C

| Enzyme | Residues per molecule (mol. wt. approximately 30,000) Phenylalanine | Tyrosine | Tryptophan |
|---|---|---|---|
| B | 11 | 8 | 6 |
| C | 12 | 8–9 | 7 |

Data from refs. 3 and 4, and from unpublished studies by Dr. Guido Guidotti in this laboratory.

tifacts, resembling Cotton effects, can arise under just such conditions. If the polarimeter is set to record the optical rotation at a wavelength which is strongly absorbed, and if the instrument is at the same time receiving stray light of a longer wavelength which is not appreciably absorbed, such artifacts may be obtained. We have therefore examined our data by determining the dispersion curves in cells of different length and with solutions of differing protein concentration, so that the absorbance of the solutions was varied from values as low as 1.3 and as high as 4.6 for carbonic anhydrase B, and over a range from 1.9 to nearly 4 for carbonic anhydrase C. The results are shown in Table 2.

TABLE 2

MOLAR RESIDUE ROTATIONS OF NATIVE HUMAN CARBONIC ANHYDRASES

| Concentration (gm/100 ml) | Cell length (dm) | $A_{280}$ | $-[R']$ at 300 mμ | Relative $[R']$ Values $= [R']\lambda/[R']$ 300 mμ at Wavelength (mμ) 297 | 293 | 289 | 265 |
|---|---|---|---|---|---|---|---|
| Carbonic Anhydrase B | | | | | | | |
| 0.561 | 0.05 | 4.57 | 412 | 1.04 | 0.98 | — | — |
| 0.561 | 0.05 | 4.57 | 401 | 1.04 | 1.00 | 1.18 | 0.93 |
| 0.333 | 0.05 | 2.72 | 404 | 1.05 | 1.00 | 1.22 | 0.95 |
| 0.161 | 0.05 | 1.31 | 406 | 1.03 | 0.97 | 1.20 | 0.89 |
| 0.161 | 0.10 | 2.62 | 397 | 1.04 | 0.97 | 1.21 | 0.89 |
| Carbonic Anhydrase C | | | | | | | |
| 0.440 | 0.05 | 3.92 | (457) | 1.03 | 1.00 | 1.18 | — |
| 0.173 | 0.05 | 1.54 | 516 | 1.02 | 1.01 | 1.15 | 1.10 |
| 0.109 | 0.10 | 1.95 | 505 | 1.01 | 0.97 | 1.15 | 1.04 |

The symbol $A_{280}$ denotes the actual absorbance at 280 mμ of the solution in the cell used for the measurements. The measured rotations at 289 mμ, for the data reported here, ranged from $-0.0353° \pm 0.0008°$ to $-0.1300 \pm 0.0066°$. The corrections for blanks ranged from $+0.0005°$ to $0.0226°$ at the same wavelength. All solutions were dissolved in 0.1 *M* phosphate buffer, pH 7.0. Temperature 25° ± 1°.

Although there is some variation from one run to another in the values of $[R']$ at a given wavelength, the relative rotations at different wavelengths, compared to the value at 300 mμ, remain constant within experimental error over the very wide range of absorbance values studied. This is true even in the case of one series of measurements on carbonic anhydrase C, enclosed in parentheses in Table 2. The absolute values of $[R']$ for this run are approximately 8–10 per cent lower than those in the other measurements reported. However, the relative values at the different wavelengths are nearly identical with those recorded in the other runs. These data appear to provide decisive evidence that the peaks and troughs shown in the curves of Figures 3 and 4 represent genuine Cotton effects, not artifacts. It is certainly in harmony with this conclusion that the Cotton effects progressively disappear as the guanidine hydrochloride concentration is increased. At 0.5 *M* guanidine hydro-

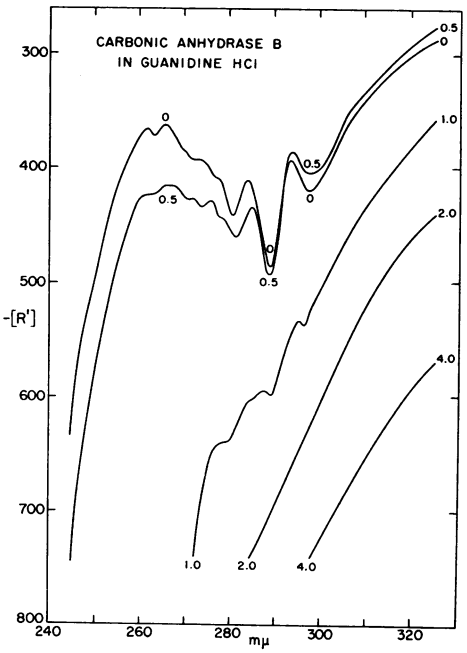


FIG. 3.—Rotatory dispersion curves between 240 and 320 mμ for carbonic anhydrase B in the native state at pH 7.0 (curve marked 0) and in guanidine hydrochloride solutions at molar concentrations indicated by the numbers adjoining the curves. All solutions contained the same 0.1 *M* phosphate buffer, which had a pH of 7.0 in water. The guanidine hydrochloride solutions had stood at 0° for 48 hr before the measurements were made.

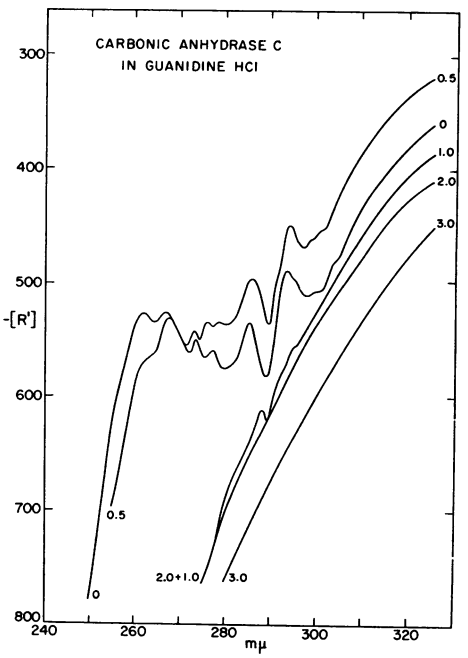


FIG. 4.—Rotatory dispersion curves for carbonic anhydrase C in aqueous buffer and in guanidine hydrochloride solutions. Conditions and symbols are identical with those of Fig. 3.

chloride the Cotton effects are nearly but not quite as pronounced as in the native proteins. At 1 *M* only small traces of these effects remain, and at 2 *M* and above, the curves are as simple and featureless as they are found to be for the acid denatured proteins in Figures 1 and 2. The effects of adding urea to the enzyme solutions, which are not shown in the figures here, are closely similar to those of guanidine hydrochloride. However, the molar concentration of urea necessary to diminish the Cotton effects to a given extent is approximately 2.5 times as great as the corresponding concentration of guanidine hydrochloride.

Samples of carbonic anhydrases B and C provided by Dr. P.-O. Nyman gave dispersion curves and Cotton effects which were within the limits of error identical with those which we have found for our own preparations. Likewise, Dr. Nyman's preparation of carbonic anhydrase A gave dispersion curves and Cotton effects very similar to those of enzyme B. This similarity is not surprising since Nyman and Lindskog[4] have shown enzymes A and B to be extraordinarily similar in amino acid composition and also in specific activity. Enzyme C differs clearly from the other two in both respects. The close agreement between the Cotton effects found for Nyman's preparations of enzymes B and C and ours is significant, since he has separated these enzymes[1] by techniques very different from ours.

Although there is a broad general resemblance between the dispersion curves for

enzymes B and C as shown in Figures 3 and 4, there are notable and reproducible differences in detail. Both the native enzymes at pH 7 show a trough in the dispersion curve at 297 mμ and a peak which lies at 293 mμ for enzyme B and at 294 for enzyme C. This difference in the position of the peak, although small, appears to be reproducible. The curves for both enzymes turn sharply downward between 293 and 289 mμ. The decrease in [R′] for enzyme B is approximately 90° between 293 and 289 mμ although in some runs it has been as high as 110°. The value for enzyme C—$\Delta[-R']$ from 294 to 289—is somewhat lower, approximately 80–85°. There is another peak near 284 for both enzymes and a shallow trough close to 281, but the change in [R′] between the peak and trough is relatively small here, approximately 20–25°. The dispersion curve for enzyme B then continues to rise, as the wavelength decreases, with a broad peak—possibly a double peak—between 260 and 265 mμ. Below 260 mμ the curve descends steeply to more negative values of [R′]. For enzyme C, on the other hand, the peak in the 260–265 region is much smaller, and the curve then descends rapidly to more negative values.

The patterns illustrated in Figures 3 and 4 are sufficiently complex to suggest that several small Cotton effects are involved. However, we do not claim that all the recorded fluctuations are significant. The amplitude of the random oscillations of the recording pen, in the region in which the enzymes show strong absorption, is markedly greater than in regions where the proteins are transparent. Because of this increase in noise level, the uncertainty in the values of [R′] may be as great as ± 20° in the region between 275 and 290 mμ. Some of the minor fluctuations in the curves of Figures 3 and 4 may therefore not be significant, but the major features are regularly reproducible.

*Interpretation of Dispersion Data at Longer Wavelengths.*—A previous paper from this laboratory[3] reported data for the rotatory dispersion of carbonic anhydrase B at wavelengths between 300 and 600 mμ, and inferred that they suggested an absence, or at least a very low content, of helix in the conformation of the peptide chain. We have since made many more measurements with both enzymes B and C, and a detailed report will be given elsewhere. We note that the analysis of these data for the native proteins, in terms of the equation of Moffitt and Yang,[17] or that of Shechter and Blout,[18] is rendered somewhat uncertain by the presence of the unusual Cotton effects reported here. Effects of this sort are not allowed for in the theory underlying these equations. The denatured proteins, in urea or guanidine hydrochloride, give data which fit the above equations satisfactorily, and also indicate a very low helix content, of the order of 10 per cent, after denaturation.

*Discussion.*—The human carbonic anhydrases, in their native state, are highly compact molecules. Enzyme B has an intrinsic viscosity between 2.7 and 2.8 ml $g^{-1}$ (refs. 19 and 20) which is one of the lowest values recorded for any protein, and clearly implies that the shape of the molecule is nearly spherical. Although precise viscosity measurements are not yet available for enzyme C, its intrinsic viscosity is certainly also very low. The sedimentation coefficients of these enzymes, $s_{20,w}$ = 3.0 S, also indicate a very compact structure in view of the molecular weights which are close to 30,000.

The titration studies of Riddiford[21, 22] show clearly that most of the histidine and tyrosine residues in the native molecules are inaccessible to the solvent and are presumably buried in the interior of the native molecule, becoming available for

titration only on denaturation. Likewise, the study of the difference spectra produced by denaturation in acid,[3, 23] particularly the difference peak at 291.5 mμ which is associated with the tryptophan residues, indicates that most or all of the tryptophan groups in the native molecules—6 in enzyme B, 7 in C—lie in the molecular interior.

This evidence is highly relevant to the interpretation of the Cotton effects between 260 and 300 mμ, shown in Figures 1–4. Although these effects are too complicated to be readily interpretable in detail, the positive Cotton effect with a peak at 293–294 mμ and a trough at 289 mμ almost certainly is associated with the tryptophan absorption band. The somewhat smaller Cotton effect with a peak near 285 and a trough close to 280 may well be associated with tyrosine interactions, although the tryptophan residues may also contribute. The pattern of the dispersion curves between 260 and 270 mμ (Figs. 3 and 4) suggests that the phenylalanine residues in the native proteins may also make small contributions to the Cotton effects in this region. These effects, however, are so small that they may not be significant, because of the noise level in the tracings made in this region. A study of the circular dichroism of the carbonic anhydrases may be extremely helpful in providing a more detailed analysis of these effects. Dr. George Holzwarth has made some preliminary studies of circular dichroism on our carbonic anhydrase preparations and has established definitely the presence of at least one negative band centered near 275 mμ. However, the resolving power of the measurements was too low to distinguish any fine structure.

The disappearance of the Cotton effects in the 260–300 mμ range on denaturation in acid, urea, or guanidine hydrochloride is strikingly shown in Figures 1–4. Presumably in the native proteins the aromatic side chains are built quite tightly into the native protein structure and buried in the interior of the molecule, adjacent to centers of optical asymmetry. It is impossible to say at present whether arrangements occur in which 2 or more aromatic rings are brought in close proximity to one another.

Tyrosine itself shows a well-defined Cotton effect; in neutral solution the peak lies near 286 mμ and the trough near 254.[24, 25] Cotton effects in poly-L-tyrosine have been studied by Fasman, Bodenheimer, and Lindblow.[25] The patterns are quite different for the helical and the random-coil forms of the polymer. Studies of circular dichroism[26] have greatly clarified the nature of these Cotton effects, and have shown the presence of positive bands at 270 and 248 mμ and a much stronger negative band at 224 mμ in the helical polymer.

However, the rotatory dispersion of the carbonic anhydrases shows a very different pattern from that of poly-L-tyrosine. Moreover, Fasman *et al.*[25] have studied copolymers of L-tyrosine and L-glutamic acid at molar ratios of 1:9 and 2:8, respectively. In the 1:9 polymer the Cotton effects completely disappear. In the 2:8 polymer they are still present but quite weak. Carbonic anhydrase B, on the other hand, contains only 6 tryptophan and 8 tyrosine residues out of a total of about 260 amino acid residues; and enzyme C contains 7 tryptophan and probably 9 tyrosine residues in a molecule of nearly the same size.[3, 4, 27] The tryptophan and tyrosine residues, added together, compose only 5–6 per cent of these molecules. The observed change in [R′] of approximately 90° between 293 and 289 mμ (Fig. 3) would correspond to a change in [R′] per tryptophan residue of the order of 4000°,

if we assume that all six tryptophan groups contribute to the observed effects. If only one of the six were involved, its contribution would be 24,000°. Even the smaller figure is substantially larger than the effects characterizing poly-L-tyrosine, for which the change in [R'] is of the order of 500° between 286 and 275 mμ.

There have been few previous reports of Cotton effects associated with light absorption by aromatic side chains in proteins. Schellman and Schellman[28] in 1956 noticed a small bulge near 290 mμ in the rotatory dispersion curve of bovine plasma albumin, and Jirgensons[29] recorded a similar effect in human albumin; but Sogami, Leonard, and Foster[30] stated that they could observe no such effect in a sample of albumin from which lipid had been carefully removed. Simmons and Blout[13] have noted a similar small peak near 290 mμ in the protein of tobacco mosaic virus, which vanished on denaturation of the protein. Grosjean and Tari,[31] in a paper describing a new instrument, incidentally reported for insulin a negative band of circular dichroism centered near 275 mμ. Presumably there should be Cotton effects corresponding to this band, which have not yet been reported.

The most striking Cotton effects in a protein, which must clearly be associated with aromatic absorption bands, are those observed by Green[32] on avidin of egg white, which contains approximately 14 tryptophan and 4 tyrosine residues[33] per molecule of molecular weight 53,000. Avidin shows two positive Cotton effects, one relatively small with a peak near 293 mμ and a trough at 256 mμ, the other much higher and steeper, with a peak at 233 mμ $[\alpha] = +1120°$, and a trough at 220 mμ, $[\alpha] = -1400°$. This Cotton effect is unique, to our knowledge; we have seen no suggestion of anything even slightly resembling it in the carbonic anhydrases.

It is quite possible that many other proteins will be found to show Cotton effects associated with the absorption bands of the aromatic side chains. The resolving power and accuracy of some of the newly developed instruments, such as the Cary instrument which we have employed, and their relative freedom from artifacts due to stray light, should permit the detection of such effects.

Further studies on the carbonic anhydrases are obviously needed. It is now readily possible to remove the zinc atom from the carbonic anhydrases with disappearance of enzyme activity, and then to add zinc and simultaneously restore activity.[34, 35] Lindskog and Malmström[36] have reported that this reversible removal of zinc from bovine carbonic anhydrase does not appreciably affect the rotatory dispersion pattern at wavelengths above 300 mμ. However, the Cotton effects in the 260–290 mμ region may be much more sensitive to minor changes in protein conformation which might accompany the removal of the zinc. Studies to examine this point are now under way in this laboratory.

*Summary.*—Carbonic anhydrases A, B, and C from human erythrocytes give unusual optical rotatory dispersion curves involving several relatively small but reproducible Cotton effects in the region between 260 and 300 mμ, associated with the absorption bands of the aromatic side chains in these proteins. When the highly compact structure of the native proteins is disrupted by denaturation in acid, urea, or guanidine hydrochloride solutions, these Cotton effects disappear. The rotatory dispersion curves for the native enzymes B and C pass through troughs at 223 and 227 mμ, respectively, with values of the molar residue rotation of the order of −2300° at this minimum.

This article was dedicated to EDWIN BIDWELL WILSON on the occasion of his fiftieth anniversary as Managing Editor of these PROCEEDINGS.

*Note added in proof:* Since submitting this manuscript, we have learned from Dr. Andreas Rosenberg of the University of Minnesota that he has independently carried out similar studies on both human and bovine carbonic anhydrases.

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